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Indian Standard

METHODS OF SAMPLING AND TEST FOR OILS AND FATS

PART III ANALYSIS BY GAS LIQUID CHROMATOGRAPHY

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Indian Standard

METHODS OF SAMPLING AND TEST FOR OILS AND FATS

PART III ANALYSIS BY GAS LIQUID CHROMATOGRAPHY

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AMENDMENT NO. 1 MAY 1986

TO

IS:548(Part 3)-1976 METHODS OF SAMPLING AND TEST FOR OILS AND FATS

PART 3 ANALYSIS BY GAS LIQUID CHROMATOGRAPHY

(*Page 4, clause 2.4, line 2*) - Substitute 'a known fatty acid ester, for example methyl palmitate or methyl heptadeconoate' *for* 'methyl palmitate'.

(*Page 5, clause 2.6, line 2*) - Substitute 'a known fatty acid ester, for example, methyl palmitate or methyl heptadeconoate' *for* 'methyl palmitate'.

(*Page 7, clause 8.1.1, heading*) - Substitute '*Stainless Steel or Glass Columns*' for the existing headings.

(CAFDC 5)

Indian Standard

METHODS OF SAMPLING AND TEST FOR OILS AND FATS

PART III ANALYSIS BY GAS LIQUID CHROMATOGRAPHY

0. FOREWORD

0.1 This Indian Standard (Part III) was adopted by the Indian Standards Institution on 25 November 1976, after the draft finalized by the Oils and Oilseeds Sectional Committee had been approved by the Chemical Division Council and the Agricultural and Food Products Division Council.

0.2 IS : 548 was first published in 1954 and subsequently revised in 1964. It covered methods of sampling, physical, chemical and qualitative tests. In view of periodic review of qualitative tests for detection of adulteration in oils and fats, the concerned technical committee decided to cover such tests in Part II of this standard and IS : 548 (Part II)-1976* was accordingly published. The methods of sampling, physical and chemical tests were covered in IS : 548 (Part I)-1964†.

0.3 Now that modern analytical techniques like gas liquid chromatography (GLC) have been well standardized and are practised by many laboratories in the country and have distinct advantages for complete and quick analysis of oils and fats as compared to other methods as covered in Part I of this standard, the concerned technical committee decided to prescribe analysis by GLC as Part III of this standard. The GLC technique is considered to be more reliable and it is felt that by adoption of this method innumerable problems in testing and detection of adulteration would be substantially solved.

0.4 In GLC procedure methyl esters prepared from the samples of oils, fats or fatty acids are dissolved in a suitable solvent and separated by GLC. A commonly used method for detecting products eluted in vapour chromatography is to note the change in the thermal conductivity of the eluted gas when a product appears. Other methods of detection include the use of flame ionization, argon ionization and gas density detectors. From the chromatographic chart obtained, fatty acid composition of the sample is calculated and compared with the reference or standard composition chart

*Methods of sampling and test for oils and fats: Part II Purity tests (*third revision*).

†Methods of sampling and test for oils and fats: Part I Methods of sampling, physical and chemical tests (*revised*).

of individual oils and fats. It is, therefore, desirable to prescribe specifications of oils and fats in terms of their fatty acid composition. This matter is under active consideration of the committee and such specifications may ultimately form a part of this standard. This method is, therefore, complementary to physico-chemical tests for analysis of oils and fats. This standard, however, does not cover detection of oils and/or fatty acids containing hydroxyl and other polar groups, for example, castor oil.

0.5 In the preparation of this standard substantial assistance has been derived from data supplied by Regional Research Laboratory, Hyderabad; Directorate of Sugar and Vanaspati, Ministry of Agriculture and Irrigation, Government of India; and the draft International Standard prepared by Technical Committee ISO/TC 34 Agricultural Food Products of International Organization for Standardization (ISO) which is thankfully acknowledged.

0.6 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1960*.

1. SCOPE

1.1 This standard (Part III) prescribes methods of sampling and analysis of oils and fats of vegetable and animal origin and also of technical fatty acids using gas liquid chromatography (GLC).

2. TERMINOLOGY

2.0 For the purpose of this standard, the definitions given under 2 of IS : 548 (Part I)-1964† and the following shall apply.

2.1 Homologous Series of Fatty Acids — A series of fatty acids of which two consecutive members differ only by a methylene group.

2.2 Carbon Number — The total number of carbon atoms present in a fatty acid. The methyl ester of a fatty acid, although it has one carbon atom more than the fatty acid is taken as the same as the fatty acid regarding carbon number.

2.3 Retention Time — Time in seconds between the moment of injection to the midpoint of the peak belonging to the component to be identified.

2.4 Relative Retention Time — It is the ratio between the retention time of a component to be identified and that of methyl palmitate.

*Rules for rounding off numerical values (*revised*).

†Methods of sampling and test for oils and fats: Part I Methods of sampling, physica and chemical tests (*revised*).

2.5 Retention Distance — The distance in millimetres in the chromatogram between the injection point and the midpoint of the peak belonging to the component to be identified.

2.6 Relative Retention Distance — It is the ratio between the retention distance of a component to be identified and that of methyl palmitate.

2.7 Equivalent Chain Length (ECL) — The ECL values are used to express the elution sequence of esters from a gas chromatographic column. These values are determined from a calibration graph obtained by plotting logarithms of the relative retention times of two or more known, normal, even number, saturated monocarboxylic methyl esters against the number of carbon atoms in the acid. ECL values of other methyl esters chromatographed under identical operating conditions are then read from the calibration graph using observed relative retention times.

3. SAMPLING

3.1 Representative samples of the material shall be drawn as prescribed in 3 of IS : 548 (Part I)-1964*.

4. QUALITY OF REAGENTS

4.1 Unless otherwise specified, pure chemicals and distilled water (*see* IS : 1070-1960†) shall be employed in tests.

NOTE — ' Pure chemicals ' shall mean chemicals that do not contain impurities which affect the results of analysis.

5. GENERAL PRECAUTIONS

5.1 Melt the sample if it is not already liquid, mix it thoroughly and filter through a filter paper to remove any impurities and the last traces of moisture.

6. OUTLINE OF THE METHOD

6.1 In GLC the moving phase is a gas. The mixture of methyl esters is vapourized into the column and fractionated by being partitioned between the moving phase and the non-volatile stationary liquid phase impregnated on a solid support. An inert gas such as helium, nitrogen or argon is used as carrier and eluant. Where high purity nitrogen or helium is not available, hydrogen is used as a carrier gas for use with thermal conductivity detector. The retention time of a particular species depends on its vapour pressure and its solubility in the immobile liquid. By varying the nature of the liquid phase, columns having a wide variety of selectivity may be prepared. For example polar liquids are most successful in

*Methods of sampling and test for oils and fats: Part I Methods of sampling, physical and chemical tests (*revised*).

†Specification for water, distilled quality (*revised*).

effecting the separation of closely related polar compounds and hydrocarbon greases are useful for the separation of esters differing only in chain length. Use of heated columns and vapourization chambers allow separation by gas chromatography of materials that vapourize at temperatures as high as 400°C. The method is commonly used for analysis of samples in milligram quantities. A commonly used method for detecting products eluted in a vapour chromatogram is to note the change in the thermal conductivity of the eluted gas when a product appears. Another common method of detection includes the use of flame ionization detector.

6.2 Methyl esters prepared from the fats or fatty acids are dissolved in chloroform and are separated by GLC. From the chart obtained, the fatty acid composition of the sample is calculated.

7. PREPARATION OF METHYL ESTERS

7.0 General — This method is suitable for the preparation of methyl esters of fatty acids from the fats and oils as well as from the technical fatty acids. It is, however, not suitable when epoxy, hydroperoxy and cyclopropenyl groups are present.

7.1 Reagents

7.1.1 *Methanolic Sodium Hydroxide Solution* — approximately 0·5 N. Dissolve 2 g of sodium hydroxide in 100 ml of methanol containing not more than 5 percent water. Store the solution in a glass stoppered conical flask. When used during a longer period of time sodium carbonate may be formed as precipitate but this does not interfere with the methyl ester preparation.

7.1.2 *Methanol* — Methanol of high purity is to be used.

7.1.3 *Petroleum Ether* — boiling point 40 to 60°C.

7.1.4 *Sodium Sulphate* — anhydrous.

7.1.5 *Sulphuric Acid* — concentrated.

7.1.6 *Hydrochloric Acid* — concentrated.

7.2 Apparatus

7.2.1 *Round Bottom Flask* — having 50-ml capacity and with ground glass joint B 24 or B 19.

7.2.2 *Water Cooled Reflux Condenser* — length 20 to 30 cm, with B 24 or B 19 ground glass joint, having a round bottom flask and matching the flask (*see* **7.2.1**).

7.2.3 *Boiling Chips, Fat-Free, or Glass Beads*

7.2.4 *Capillary Pipettes*

7.2.5 *Graduated Pipette* — 10 ml capacity.

7.2.6 *Glass Stoppered Test Tubes*

7.2.7 *Separating Funnels* — 100 ml capacity.

7.3 Procedure

7.3.1 *Weighing* — Prepare the sample as prescribed under **5.1** and homogenise. Transfer approximately 150 mg of this sample into a round bottom flask.

7.3.2 *Preparation of Methyl Esters from Oils and Fats* — Add 5 ml of methanolic sodium hydroxide solution and a few boiling chips or glass beads in the round bottom flask containing sample. Connect the flask to the reflux condenser and boil the mixture under reflux for half an hour. At the end of this period remove the flask from the reflux condenser and evaporate a little methyl alcohol. Add to this 5 ml of water containing 1 ml of concentrated hydrochloric acid and extract with 10 ml of petroleum ether. Repeat the extraction with petroleum ether two times more. Combine the petroleum ether solutions, wash with water twice and dry with 5 g of anhydrous sodium sulphate. Remove the petroleum ether preferably using a rotary vacuum evaporator and/or in a current of nitrogen. Add 10 ml of pure methyl alcohol and 2 drops of concentrated sulphuric acid to the free fatty acids thus obtained. Reflux the mixture for 3 hours. At the end of this period dilute the mixture with 10 ml of water and extract with 10 ml of petroleum ether. Repeat the extraction 2 times more with 10 ml of petroleum ether. Combine the petroleum ether extracts, wash with water twice and dry with anhydrous sodium sulphate. Remove the petroleum ether as before. The methyl esters are now ready for GLC analysis.

7.3.3 *Preparation of Methyl Esters from Fatty Acids* — When the sample contains only fatty acids the saponification step is omitted. Add directly 10 ml of methyl alcohol and 2 drops of concentrated sulphuric acid and follow the procedure as prescribed in **7.3.2** to get the methyl esters.

8. APPARATUS

8.1 Gas Liquid Chromatograph — consisting of the parts prescribed in **8.1.1** to **8.1.5**, **8.2**, **8.4** and **8.5** as supplied by the manufacturer.

8.1.1 *Copper, Stainless Steel, Glass or Aluminium Columns* — with internal diameter of about 3 to 6 mm and 2 to 2·5 m length.

8.1.2 *Column Packing* — consisting of:

a) *Solid support* — Diatomaceous earth acid washed and silanized suitable for gas chromatography (for example, Chromosorb W, Gas chrom Q and Anachrom ABS) of 60 to 80, 80 to 100 or 100 to 120 mesh,

b) *Stationary Phase* — Diethylene glycol succinate (DEGS) or ethylene glycoladipate (EGA) polyester or other suitable stationary phase.

c) *Recommended mass ratio of stationary phase to solid support* — 1 : 6 to 1 : 5.

8.1.2.1 *Preparation of the packing material* — Dissolve the stationary phase in a suitable solvent, such as acetone or chloroform. Add this solution to the solid support. The solid support shall be completely covered by the liquid. Shake the mixture thoroughly. Remove the solvent preferably using a rotary vacuum evaporator. Dry the material at 105°C for three hours and keep it in a glass stoppered bottle until use.

8.1.2.2 *Packing of column* — Fill the column with the impregnated material while continuously and gently tapping with a plastic hammer along the entire column. Packing of the column under suction (water pump or even oil pump for fine supports) is advised. The empty column and the completely filled column are weighed and the difference in mass is noted. Normally, a 2·5 m long × 5 mm diameter column packed with diethylene glycol succinate on Chromosorb would take about 15 g of the material. Put plugs of glass wool on both ends.

8.1.3 *Injection Port* — With controllable heating, preferably suitable for direct injection of the column.

8.1.4 *Detector and Convertor Amplifier* — The use of a flame ionization detector is recommended to obtain the highest possible degree of sensitivity and linearity. Thermal conductivity detector is less sensitive than flame ionization detector.

8.1.5 *Gas Flow Regulator* — Gas flow regulating unit for carrier gas (hydrogen or helium in the thermal conductivity unit and nitrogen, hydrogen and air for the flame ionization detector) is required to obtain steady flow rate of gases. The flow rate of the carrier gas shall be measured with a soap bubble flow meter and shall be constant up to ± 2 percent of the set value. Normally, a flow rate of 40 to 60 ml/min carrier gas is used.

8.2 Recorder — A recorder which can be adapted to the converter amplifier or katharometer is required. Normally, a 1 mA/s recorder is useful. A 3 mA/s unit may also be used. Often, an electronic integrator or a computer can be hooked on to the recorder.

8.3 Gases — The carrier gas shall be very pure and dry. If nitrogen is used, the oxygen content in it shall be less than 5 ppm. This may be achieved by passing the gas over a heated copper catalyst after which it shall be dried using a molecular sieve of 5 Å mesh size. Hydrogen gas of 99·9 percent purity is required. Organic material and water shall be absent. In the air sample used for flame ionization detection, organic material shall be absent.

8.4 Supply Lines and Connections — Supply lines and connections shall be made of a material which may not give interfering products. Suitable materials are tempered copper, nylon and teflon.

8.5 Syringe for Injection of Sample — The volume shall not exceed 10 µl and graduations of 0·2 µl or less shall be used.

9. PROCEDURE

9.1 Adjustment of the Apparatus — Check the base line adjustment of the recorder and convertor amplifier or katharometer power supply according to the instructions given by the manufacturer. Instrument should be free from gas leakage when connections are made.

9.1.1 *Conditioning of the Column* — Disconnect the carrier gas inlet from the column to the detector. Raise the temperature of the thermostatically controlled oven to 185°C during the course of 4 hours with a constant carrier gas flow of 30 to 40 ml/min. Keep the column at this temperature for at least 16 hours. The column might shrink during this process. If this be the case, add some fresh column packing and repeat the conditioning procedure for an additional 16 hours. Finally, heat for 2 hours at 195°C at the same carrier gas flow. Occasionally columns are conditioned even for 48 hours to obtain a satisfactory base line.

9.1.2 *Gas Flow* — Normally a flow rate of 40 to 60 ml/min is chosen in order to obtain optimum separation. When flame ionization detector (FID) is used the flow rate of hydrogen gas shall be about half the rate of the carrier gas; the rate of air will be 5 to 10 times the rate of hydrogen. Most of the information given above is provided in the manual supplied along with the gas chromatographic apparatus.

9.1.3 *Determination of Optimum Working Conditions* — The following variables are involved in choosing the working conditions:

a) Column length and diameter,
b) Amount and type of stationary phase and solid support,
c) Temperature of the column,
d) Rate of flow of the carrier gas,
e) Resolution required,
f) Sample size, and
g) Time of analysis.

9.1.3.1 This standard is based on a column length of 2 to 2·5 m with a maximum inside diameter of 5 mm and with 15 percent diethylene glycol succinate (DEGS) polyester. The resolution required will be at least 4 000 theoretical plates and a speed of analysis at which methyl stearate is eluted in about 15 minutes.

9.1.3.2 The other variables that remain are flow rate of carrier gas, column temperature, sample size and to a small extent the diameter of the column, the amount of stationary phase and the type of support. These variables shall be chosen to obtain a good resolution. Normally 250 cm × 5 mm column packed with 15 percent DEGS on Chromosorb W, 45 to 60 or 60 to 80 mesh is used in analysis of methyl esters of all the common fatty acids.

9.1.4 *Determination of Resolution and Selectivity* — Set the temperature of injection port at about 230°C. The detector temperature shall be at least equal to column temperature or slightly more (about 10 deg C). Inject into the column a mixture containing approximately equal amounts of methyl oleate and methyl stearate dissolved in a suitable solvent. Select the amount of mixture, flow rate of the gas recorder attenuator and the column temperature in such a way that maximum of the methyl stearate peak is recorded at least 15 minutes after the solvent peak and the height of the methyl stearate peak is about 60 percent of the paper width. The oleate peak shall be well resolved from the stearate peak. The chart speed shall be at least 75 cm/h.

9.2 Amount of Sample — Normally with thermal conductivity detector a sample of 0·5 to 1·0 µl without solvent gives a fairly good chromatogram. For small peaks, during the analysis, higher attenuation can be used and the new base line is recorded. When working with flame ionization detector, dilute samples shall be used.

9.3 Injection of the Sample — After drawing the sample into the syringe, the outer surface of the needle is wiped off with clean tissue paper. The needle is introduced into the injection chamber fully and quickly. After injection the needle is withdrawn immediately.

9.4 Temperature — For isothermal runs of methyl esters on a polyester column, a temperature of 185 to 190°C is recommended. For temperature programmed runs of mixtures containing esters of widely varying chain length, it is advisable to start from a low temperature, say anywhere from 60 to 100°C, and run the analysis at the rate of 2 to 5 deg C per minute till 195°C is reached and isothermally thereafter. The temperature of the injection port would be around 230°C and that of the detector block at 210°C. For temperature programmed runs, a dual column, dual injection port, dual detector gas chromatograph is advised.

9.5 Chart Speed — If the chart is used for quantitative interpretation, an accurate adjustment of the chart speed is important. Choose the speed of the recorder in such a way that the width at half height of peaks covering about 10 percent or more of the total peak area is at least 10 mm.

10. CALCULATION

10.1 Qualitative Interpretation

10.1.1 *General* — When chromatographing a mixture of methyl esters the first peak which appears on the chromatogram is that of the solvent. The components of the sample to be investigated are eluted according to ascending molecular mass or number of carbon atoms, the unsaturated esters invariably leaving the column after the corresponding saturated compounds.

In the case of unsaturated components the number and the position of double bonds influence the retention time depending on the polarity of the stationary phase. Generally with an increasing number of double bonds per molecule and a constant number of carbon atoms, the retention time increases; also conjugation gives a longer retention time. On highly polar stationary phase *cis* and *trans* isomers are generally not separated. They elute as a single peak. Branching of straight chain gives shorter retention time. Critical pairs (such as 18 : 1 & 20 : 0 and 20 : 1 & 22 : 0) of methyl esters with different chain lengths and varying degrees of unsaturation but eluting together are also encountered. In many cases, it will be possible to identify every peak with the information given above. In the case of peaks which cannot be identified with certainty their carbon number has to be determined. In such cases isothermal runs are recommended as there is a linear relationship between the chain length and logarithms of the retention time in a conformable series of fatty acid methyl esters.

10.1.2 *Test Mixture* — This is a mixture of methyl esters of unbranched saturated fatty acids, the composition of which is qualitatively known. It can be used for identifying a mixture of fatty acid methyl esters. The following mixture is recommended. The proportions are :

a) Methyl laurate (12 : 0) 5 percent,

b) Methyl myristate (14 : 0) 5 percent,

c) Methyl palmitate (16 : 0) 15 percent,

d) Methyl stearate (18 : 0) 25 percent,

e) Methyl oleate (18 : 1) 25 percent,

f) Methyl linoleate (18 : 2) 15 percent, and

g) Methyl arachidate (20 : 0) 10 percent.

10.1.3 *Procedure* — Using a mixture mentioned in **10.1.2** prepared either in the laboratory or purchased from a reliable source, prepare a chromatogram of it working isothermally at 185°C. Measure the retention distance of all peaks accurately.

10.1.4 *Carbon Number* — The retention distances of the peaks belonging to the test mixture are plotted (on a logarithmic scale) against their carbon

number (on a linear scale). A straight line is drawn from points thus obtained. If one or more points deviate visibly from the line the determination shall be repeated. From this graph the carbon numbers of the unknown peaks can be ascertained from their retention times.

10.1.5 *Other Methods for the Identification of Unknown Peaks*

10.1.5.1 *Calculation of relative retention times with respect to methyl palmitate* — This method is more suitable if the working conditions are identical. The disadvantage of this method is that a total of all relative retention times of all methyl esters with respect to methyl palmitate should be available.

10.1.5.2 *Plotting logarithmic retention time against number of carbon atoms* — This method makes use of the fact that on plotting logarithmic retention time against the number of carbon atoms of fatty acid, methyl esters which belong to a homologous series lie in a straight line. However, for various homologous series these lines run parallel.

10.2 Quantitative Interpretation

10.2.1 *General*

10.2.1.1 *Base line* — The base line of chromatogram is the line which the recorder makes when no material is present in the eluant gas. If spurious signals are present in the chromatogram before the sample is injected, it means that there is some variation in temperature of the oven or in the case of ionization detector system, the electrometer may not be working properly. Improper conditioning of the column or leakage through the septum in the injection port can also cause difficulties. Deviations in the base line may be due to noise or wave motions or drift and should be corrected by attending to the electronic parts of the instrument. In such cases, the base line of the peak is drawn manually connecting the beginning and end points of the peak by a straight line.

10.2.1.2 *Peak height* — Sample size to be injected shall be chosen in such a way that the peak height of the component present to the maximum extent covers at least 75 percent or utmost 85 percent of the available paper width. The smallest component to be calculated quantitatively shall have a peak height of at least 1 to 2 cm.

10.2.1.3 *Symmetry of the peaks* — The GLC peaks shall preferably be symmetrical (Gaussian curve). In practice, however, this requirement is not satisfied completely as the peak often displays tailing effect. Such peaks can still be used for quantitative purposes.

10.2.2 *Calculation*

10.2.2.0 *Choice of the method of calculation* — The manner in which peak areas are calculated depends on the accuracy which is required for analysis. Among the several methods available, the following are widely used:

a) Triangulation,

b) Planimetry,

c) Disc Integrator, and

d) Electronic integrator.

10.2.2.1 *Triangulation* — For symmetrical peaks area of a peak can be calculated from the peak height and its width at half height. For others an isosceles triangle is drawn before the measurement. For incompletely separated peaks the procedure is slightly different. If the two peaks have separated to the extent of 90 percent the same procedure is employed excepting that in the portion where the two peaks are meeting, the area in that zone is divided equally between the two peaks. In the case of narrow and long peaks considerable errors may occur if the thickness of the written line on the chromatogram is not considered. Peak width shall then be measured between the outside of the ascending line of the peak and the inside of the descending line.

10.2.2.2 *Planimetry* — This method is used to calculate the absolute peak areas. It is slightly less accurate and takes more time. Unless one is very skilled reproducible results can be obtained only with difficulty. Disc integrator and electronic integrator give areas directly and are more accurate.

10.2.2.3 *Calculation and correction factors* — For accurate determinations, it is necessary to apply calibration factors in order to convert the ratios of the peak areas into mass ratios. This can be done using a test mixture of methyl ester of fatty acids. The components of the mixture shall have a high purity (about 99·0 percent). The mixture is diluted with chloroform and is injected on to the column. From the gas chromatograhic chart, peak areas of different substances are calculated and expressed in percentages. Known compositions of the fatty acid mixture are used for calculation of the correction factors.

10.2.2.4 *Calculation of the composition of the sample* — This may be done by totalling up the corrected areas of individual peaks and expressing them as percentages. Often an internal standard, a fatty acid ester not originally present in the sample to be examined which gives a peak not too far removed but well separated from the other components, is used. For this purpose methyl heptadecanoate is useful. The amount of internal standard shall be such that the area of its peak appearing in the chromatogram is approximately equal to that of the major peak in the chromatogram. For all practical purposes the corrected area percent composition obtained from the gas chomatography can be regarded as percent by mass.

11. PRECISION

11.1 Repeatability — The difference between the results of two determinations carried out on the same day with a single apparatus by the same

person, may, as far as components are concerned that occur to the extent of at least 5 percent, not differ by more than 3 percent of the value determined.

11.2 Reproducibility — The difference between the results of two determinations carried out in different laboratories may, as far as components that occur in quantity of at least 5 percent, not differ by more than 10 percent of the value determined.

12. REPORT

12.1 The results of analysis have to be reported in the following report form. In case of arbitration the chromatogram shall be attached to the report.

REPORT

Fatty Acid Composition (as Methyl Ester)

No. of analysis

Sample

Description :

Origin :

Reference :

Pretreatment of sample (methyl ester prepared from the oil or fat sample) :

Date of analysis

Description of apparatus and conditions applied :

Manufacturer and type of equipment :

Column glass, stainless steel, copper, or aluminium	GASES
Length..... m, inside diameter.....mm Reference column	Carrier gas flow.....ml/min Auxiliary hydrogen flow.....ml/min Auxiliary air flow.....ml/min
Stationary phase : Stationary phase/solid support ratio	DETECTION
Solid support Pretreatment of solid support : acid washed-silanized	Katharometer F.I.D. Attenuation of convertor amplifier :

Resolution (No. of theoretical plates to methyl stearate ....(180°)	TEMPERATURES
	Injection block...........°C Detector......C°
RECORDER	Column entrance.........°C Exit.........°C Programming.............°C/min

Chart speed.................cm/min Bridge current.......................mA

Quantitative Calculation

Triangulation/ball and disc/electronic integration, internal standard :

RESULTS (Fatty acid composition as mass percent of methyl ester)

No. of Peak	*Retention Time/ Distribution*	*Name of Fatty Acid with Symbol*	*Peak Area*	*Correction Factor*	*Corrected Area*	*Mass Percent Methyl Ester*

Total

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